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Effect of different modified atmospheric packaging (MAP) gaseous combinations on *Campylobacter* and the shelf-life of chilled poultry fillets

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ABSTRACT

Studies were undertaken to investigate the effect of different modified atmospheric packaging (MAP) gaseous combinations on *Campylobacter* and the natural microflora on poultry fillets. Skinless chicken fillets were stored in gaseous mixtures of 10%, 30%, 50%, 70% and 90% CO_2 balanced with N_2, 80:20% O_2:N_2 and 40:30:30% CO_2:O_2:N_2 and control conditions (air) at 2 °C. Samples were analysed periodically for (previously inoculated) *Campylobacter*, total viable counts (TVC) (mesophiles), TVC (psychrophiles), *Enterobacteriaceae*, *Pseudomonas* and lactic acid bacteria (LAB) over 17 days of storage. The carbon dioxide solubility was determined by monitoring the changes in the headspace volume over time using a buoyancy technique and performing calculations based on volumetric measurements and the Henry's constant. Henry's constant was also used to estimate the oxygen solubility in the chicken fillets. The presence of O_2 in the MAP gaseous mixtures increased the rate of *Campylobacter* decline on poultry fillets but in general the counts obtained in aerobic versus anaerobic packs were not significantly ($P > 0.05$) different. CO_2 inhibited the growth of TVC, TEC, LAB and *Pseudomonas* but only at MAP gaseous combinations containing 50–90% CO_2 where concentrations of up to 2000 ppm CO_2 were recorded in the fillets after 5 days. Under these conditions a shelf-life in excess of 17 days at 2 °C was obtained. Although, dissolved O_2, at levels of 33 ppm in 80:20% O_2:N_2 packs after 3 days, reduced *Campylobacter*, it also favoured the growth of the other microbes on the chicken. The optimum gaseous mixture for achieving the combined objectives of reducing *Campylobacter* and extending shelf was therefore 40:30:30 CO_2:O_2:N_2, which achieved a shelf-life in excess of 14 days.

1. Introduction

Poultry is an important source of *Campylobacter*, the primary cause of bacterial gastroenteritis in the developed world. The most recent European Food Safety Authority (EFSA) baseline survey reported a 98.3% *Campylobacter* prevalence on raw poultry carcasses in Ireland (EFSA, 2010). Although, biosecurity measures on broiler farms and interventions throughout the slaughter plant are continually being addressed, levels of *Campylobacter* contamination remain high (Haughton et al., 2010). To date, much research has focused on pre-harvest and processing interventions with few investigations on the use of modified atmospheric packaging (MAP) to control *Campylobacter* and extend shelf-life (Byrd et al., 2011). Furthermore, in the few studies that have been published, no data is provided on the amount of oxygen (O_2) and carbon dioxide (CO_2) dissolved in the product.

Poultry is a highly perishable food which deteriorates after 4–10 days post slaughter even under chilled conditions (Jimenez et al., 1997; Patsias et al, 2006a,b). MAP is a well established method to extend the shelf-life of fresh and processed chilled foods (Charles et al., 2006; Devlieghere et al., 1998; Devlieghere and Debevere, 2000; Rotabakk et al., 2010) and is used to prolong the shelf-life of poultry fillets by suppressing aerobic spoilage bacteria such as *Pseudomonas* (Sade et al., 2013). However, psychrotrophic

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facultative anaerobic bacteria such as LAB, that are less sensitive to CO_2, will grow under MAP conditions. *Enterobacetriaceae* will also grow in the presence of CO_2 but to a lesser extent than LAB (Sade et al., 2013). Data on the effect of different MAP gaseous combinations on the survival of *Campylobacter* on poultry fillets is limited (Byrd et al., 2011) and the optimum gaseous combination in terms of the elimination of *Campylobacter* while maximising shelf-life is unknown (Patsias et al., 2006b). In theory high O_2 concentrations should inhibit *Campylobacter* spp. which are microaerobic and grow optimally at about 5% O_2 and this has been demonstrated by Byrd et al. (2011). However, O_2 supports the growth of other bacteria including spoilage organisms like *Pseudomonas* and lactic acid bacteria (LAB) which produce slime, souring and/or off-odours when counts reach 10^{7-8} CFU/g (Nychas et al., 2008; Charles et al., 2006).

MAP is a non-thermal method of food preservation that uses 3 gases; nitrogen (N_2), Oxygen (O_2) and carbon dioxide (CO_2). N_2 is an inert gas with no antimicrobial activity but the anoxic atmospheres created when using this gas will select for anaerobic, aerotolerant *Lactobacilli* (Thippareddi and Phebus, 2007). Its primary function is as a filler and to prevent pack collapse. O_2 inhibits the growth of anaerobic bacteria but the principle, antimicrobial effect is due to the presence of CO_2. Although the use of CO_2 enriched modified atmospheres to extend shelf-life has been well documented (Gill et al., 1990), data for use in food safety risk assessments and shelf-life modelling studies are scarce. CO_2 readily passes through the bacterial cell membranes and four possible bacteria related mechanisms for inhibition have been suggested including: [1] the formation of carbonic acid within the bacterial cell resulting in decreased intracellular pH and reduced enzyme activity (Wolfe, 1980); [2] specific inhibition of decarboxylating enzymes (King and Nagel, 1975); [3] non-specific inhibition of susceptible non-decarboxylating enzymes (Ranson et al., 1960) and [4] alteration of membrane properties that inhibits membrane functions (Sears and Eisenberg, 1961).

Regardless, the bacteriostatic effect of CO_2 within MAP is primarily influenced by CO_2 absorption into the food and several studies have measured the solubility of CO_2 in perishable foods (Jakobsen and Bertelsen, 2004, 2006; Jakobsen and Risbo, 2009; Rotabakk et al., 2010), although poultry data is lacking. Different methods have been used in these studies including a comparison of initial and final pressures (Devlieghere and Debevere, 2000) and modified titration (Gill, 1988) but continuous monitoring requires a non-destructive approach such as the buoyancy force based method (Rotabakk et al., 2007). A minimum head space concentration of 20–30% is required to achieve bacterial inhibition (Stiles, 1991a,b) and the poultry industry therefore typically uses 40–100% CO_2 balanced with N_2.

The objectives of this study were to investigate the effect of different gaseous combinations on inoculated *Campylobacter* and the natural microflora on chilled poultry fillets and to characterise the different MAP treatments in terms of dissolved carbon dioxide and oxygen in chicken fillets throughout a given storage period.

2. Materials and methods

2.1. Culture preparation

Five *Campylobacter* strains, two strains of *Campylobacter jejuni* (1136DF, 11168 NCTC) and three strains of *Campylobacter coli* (2124GF, 323BC, 1354 DF) were used in this study. Strains were stored at −80 °C on ceramic beads (TSC, Heywood, UK). Inocula were prepared separately by aseptically transferring a bead from the stock cultures to 30 ml Hunts broth (Nutrient broth (Oxoid, Basingstoke, UK) and Yeast Extract (Oxoid, Basingstoke, UK), 5% lysed horse blood and 0.4% *Campylobacter* growth supplement FBP) and incubating at 42 °C for 48 h under microaerobic conditions (Biomerieux, Marcy l'Etoile, France). From the resultant suspension, 1 ml of each was used to inoculate 5 × 100 ml Hunts broth and these were incubated under microaerobic conditions at 42 °C for a further 24 h. Cells were recovered by centrifugation (10 min at 2655*g*), washed 3 times in maximum recovery diluent (MRD; Oxoid Basingstoke, UK), resuspended in 10 ml MRD, mixed and the volume of MRD increased to 500 ml, which gave a cell suspension containing approximately 8 $\log_{10}$ CFU/ml. Cell suspension concentrations were assessed by preparing 10 fold dilution series and plating 0.1 ml dilutions onto modified charcoal cefoperazone deoxycholate agar medium (mCCDA, Oxoid, Basingstoke, UK) plates in duplicate.

2.2. Sample preparation

Three hundred and forty chicken breast fillets were collected from the poultry processing plant immediately after chilling. The samples were transported to the laboratory at 4 ± 1 °C. They were then divided into 3 groups, group 1 and 2 each containing 160 fillets and group 3 containing 20. Group 1 and 3 fillets remained uninoculated while the 160 fillets in group 2 were inoculated using the *Campylobacter* cocktail prepared above. Each fillet was independently immersed in the freshly prepared *Campylobacter* suspension for 15 s and left at room temperature for 15 min to allow for bacteria adhesion. Using this method each fillet was inoculated with approximately 4.5 $\log_{10}$ *Campylobacter* per g. The weights of all samples were taken prior to packaging.

Groups 1 and group 2 fillets were each divided into 8 groups (labelled A to H) of 20 samples each. Group 1A and 2A samples were used as the control and packaged in air. Groups B to H had the following gaseous combinations; (B) 90/10% (N_2:CO_2), (C) 70:30% (N_2:CO_2) (D) 50:50% (N_2:CO_2) (E) 30:70% (N_2:CO_2) (F) 10:90% (N_2:CO_2) (G) 80:20% (O_2/N_2) or (H) 40:30:30% (CO_2/O_2/N_2). At times 1, 2, 3, 4, 5, 7, 9, 11, 14 and 17 days 2 samples were removed from groups 1 and 2 (eg. 1A 2A, 2B, etc.), the gaseous composition was analysed (see Section 2.6) prior to microbiological analysis (see Section 2.4). Group 3, two packs were removed from each group immediately after packaging and used repeatedly for volumetric analysis over the 17 days (see Section 2.5).

2.3. Packaging and storage

All samples were packed into EVOH semi-rigid trays 110 × 150 × 46 mm (Versatile Packaging, Ireland), gases were pumped in and heat sealed using a MECAPAC 500 MAP machine (Mecaplastic Bagnolet, France). The trays (had an oxygen transmission rate of 0.15 cm^2/Pck d bar) were covered with a 76 mm antifog high barrier film with an oxygen transmission rate of 0.8 ml/m^2/24 h (at 23 °C, 0% RH) and packed in a refrigerated room environment (<4 °C). During packaging the air was removed and flushed with food grade gas mixtures of CO_2 and N_2 (10%, 30%, 50%, 70%, 90% CO_2, balanced with N_2), 80:20% O_2:N_2 and also 40:30:30% CO_2:O_2:N_2 (BOC, Ireland). The gas/product ratio was 5:1. All samples were stored at 2 °C without light exposure for up to 17 days.

2.4. Microbiological analysis

All chicken samples in group 1, i.e., uninoculated control group, were analysed immediately for *Campylobacter* spp., total viable counts (TVC (mesophiles, 30 °C)), TVC (psychrophiles, 6.5 °C), total *Enterobacteriaceae* counts (TEC), lactic acid bacteria (LAB) and *Pseudomonas* spp. and on days 1, 2, 3, 4, 5, 7, 9, 11, 14 and 17. Group 2 samples were only analysed for *Campylobacter* spp. For microbial

enumeration, approximately 10 g of chicken fillet samples (3 × 3 cm^2 of the surface and 1 cm thickness) were weighed out aseptically, transferred to a sterile stomacher bag with 90 ml MRD (Oxoid) and pulsified for 15 s (Lab Blender 400 series, Steward Medical, London, UK). Ten fold serial dilutions of chicken homogenate were prepared and 1.0 or 0.1 ml was spread on the surface of the appropriate media for enumeration of the different bacteria.

Campylobacter spp. counts were enumerated on *Campylobacter* blood-free agar base (modified charcoal cefoperazone deoxycholate agar [mCCDA] Oxoid) and incubated at 42 °C for 48 h under microaerobic conditions (Biomerieux, Marcy l'Etoile, France) (5% O_2, 10% CO_2 and 85% N_2) in duplicate. Aerobic TVC mesophiles and psychrophiles were both enumerated on standard plate count agar (SPCA, Oxoid, Basingstoke, UK) incubated at 30 °C for 72 h and 6.5 °C for 10 days, respectively. Total enterobacteriaceae counts (TEC) were enumerated on violet red bile glucose agar (VRBGA, Oxoid, Basingstoke, UK) after incubation at 37 °C for 24 h. Lactic acid bacteria (LAB) were enumerated on De Man Rogasa Sharpe agar (MRS, Oxoid, Basingstoke, UK) following incubation at 30 °C for 72 h. Finally, *Pseudomonas* spp. were enumerated on cetrimide agar with selective supplement (CFC, Oxoid, Basingstoke, UK) after incubation at 25 °C for 48 h.

2.5. Headspace volume assessment

The headspace volumes were measured on each of the test days by submerging the packages with the product under water and measuring the resultant force with a texture analyser (Stable Micro System Ltd., TAXT2i Texture Analyser, UK), as described by Rotabakk et al (2007). Briefly, the package was submerged, upside down to avoid capture of air bubbles, at a rate of 2 mm/s for 15 s, and held submerged for 30 s to stabilize. An average of buoyancy measurements taken at 26 s, 28 s and 30 s were used in Equation (1). Atmospheric pressure at the specific time and sample date were collected from the Irish Meteorological Service and the pressures were adjusted accordingly (Glasnevin Hill, Dublin, Ireland), www.met.ie.

The package headspace volume changes were then used to calculate the absorbed CO_2 concentration in the product (Rotabakk et al., 2007)

$$C_{CO_2}^{t=\infty} = \frac{1000 \cdot P \cdot \left(V_g^{t=o} - V_g^{t=i}\right) \cdot Mw_{CO_2}}{R \cdot T \cdot W_f} \quad (1)$$

Where $C_{CO_2}^{t=\infty}$ is the total absorbed CO_2 (ppm) in the product, P is the absolute gas pressure (Pa), $V_g^{t=0}$ is initial the gas volume (m^3), $V_g^{t=i}$ is the gas volume at sampling time i (m^3), Mw_{CO_2} is the molecular weight of CO_2 (44.01 g/mol), R is the gas constant (8.314 J/mol/K^{-1}), T is the absolute temperature (K) and W_f is the weight of product (kg). After 3–4 days MA packages has shown to reach equilibrium (Sivertsvik et al., 2004; Sivertsvik and Jensen, 2005; Rotabakk et al., 2010; Fletcher et al., 2004), hence after day 3 the total amount of absorbed CO_2 was calculated according to Henry's law and the head space gas analysis.

2.6. Headspace gas analysis

The headspace gas composition (O_2 and CO_2 (ml/100 ml)) in all packs was measured immediately after packing and at each sampling time using an oxygen and carbon dioxide analyser (Checkmate 9900 analyser, PBI-Dansensor, Ringsted, Denmark). An aliquot of the headspace gas was collected with a syringe after piercing the film cover. According to Henry's law, once the package has reached equilibrium, the amount of surrounding CO_2 in the headspace is proportional to the absorbed CO_2 in the product (Rotabakk et al., 2010):

$$P_{CO_2}^{t=i} = H_{CO_{2,p}} \times C_{CO_2}^{t=i} \quad (2)$$

Where $P_{CO_2}^{t=i}$ is the partial pressure of CO_2 in the headspace (Pa), H_{CO_2} is the temperature dependent Henry's constant for CO_2 (Pa/ppm), and $C_{CO_2}^{t=i}$ is the CO_2 concentration dissolved into the product (mg/kg). Assuming that CO_2 and O_2 dissolves mainly in the water phase, one can adjust the Henry's constant for CO_2 (Carroll et al., 1991) and O_2 (Prini and Crovetto, 1989) in water to the water content in the chicken fillets resulting in 41.9 Pa/ppm for CO_2 and 2263 Pa/ppm for O_2 at 2 °C.

The O_2 solubility in the product was measured using Equation (3).

$$P_{O_2}^{t=i} = H_{O_{2,p}} \times C_{O_2}^{t=i} \quad (3)$$

Where $P_{O_2}^{t=i}$ is the partial pressure of O_2 in the headspace (Pa), H_{O_2} is the temperature dependent Henry's constant for O_2 (Pa/ppm), and $C_{O_2}^{t=i}$ is the O_2 concentration dissolved into the product (ppm).

2.7. Statistical analysis

All bacterial counts obtained from each sample were averaged and converted to $\log_{10}$ CFU/g. All experiments were repeated on 3 separate occasions. A least significant difference analysis was performed using GENSTAT ver. 12.1 (VSN International Ltd, Hemel Hempstead, UK).

3. Results

On day 0 the mean *Campylobacter* (inoculated), TVC mesophile, TVC psychrophile, TEC, LAB and Pseudomonas counts were 4.4, 2.61, 3.1, 1.64, 1.58 and 2.4 $\log_{10}$ CFU/cm^2, respectively.

Campylobacter counts for all treatments were reduced during the 17 days of storage (Table 1). Although the decline was fastest in the control (air) and other gaseous combinations that included O_2 (80:20% O_2:N_2 and 40:30:30% CO_2:O_2:N_2) these differences were not statistically significant ($P > 0.05$) with the exception of 50:50% N_2:CO_2 versus 80:20% O_2:N_2 (9 days), 50:50% N_2:CO_2 versus 80:20% O_2:N_2 and the control (11 days) and 70:30% N_2:CO_2 versus the control after 17 days.

In contrast to *Campylobacter*, TVC mesophile counts increased throughout the 17 days storage (Table 2). After 4 days the counts obtained with 10:90% N_2:CO_2 and 30:70 N_2:CO_2 were significantly ($P > 0.05$) less than the control and the 80:20% O_2:N_2 count. One day later the 50:50% N_2:CO_2 treated fillets were also significantly lower than the control and by 7 days, all treatments with the exception of 90:10% N_2:CO_2 and 80:20% O_2:N_2 demonstrated significantly ($P < 0.05$) reduced growth. This pattern was maintained until 14 days when the latter was the only treatment that was statistically similar to the control. By 17 days there was an approximate 5 $\log_{10}$ CFU/cm^2 difference between the control and any of the MAP treatments that contained 50% or higher CO_2.

A similar pattern was observed with the TVC psychrophile counts (Table 3). After 4 days, MAP treatments that contained 50% or higher CO_2 showed significantly ($P < 0.05$) reduced growth as compared to the control. These were joined by 70:30% N_2:CO_2 after 7 days and 90:10% N_2:CO_2 after 14 days. Interestingly, the 40:30:30 CO_2:O_2:N_2 combination showed significantly less growth as compared to the control after 7 days and by 17 days was approximately 4 $\log_{10}$ CFU/cm^2 lower.

Table 1
Mean *Campylobacter* counts (log_{10} CFU/cm^2) on chicken fillets packed in different MAP gaseous combinations over the course of 17 days storage at 2 °C.

Treatment/storage (days)	*Campylobacter* counts (log_{10} CFU/cm^2)									
	1	S.E[a]	2	S.E	3	S.E	4	S.E	5	S.E
Control (air)	$4.25^{A/AB}$	0.29	$4.28^{A/A}$	0.26	$4.17^{A/AB}$	0.27	$3.98^{A/ABC}$	0.30	$3.88^{A/BC}$	0.24
10:90 N_2:CO_2	$4.23^{A/AB}$	0.22	$4.23^{A/AB}$	0.26	$4.32^{A/A}$	0.28	$4.09^{A/AB}$	0.19	$4.08^{A/AB}$	0.21
30:70 N_2:CO_2	$4.43^{A/A}$	0.32	$4.27^{A/AB}$	0.22	$4.30^{A/AB}$	0.29	$4.20^{A/AB}$	0.29	$4.20^{A/AB}$	0.27
50:50 N_2:CO_2	$4.37^{A/A}$	0.26	$4.19^{A/AB}$	0.21	$4.12^{A/AB}$	0.10	$4.15^{A/AB}$	0.12	$4.23^{A/AB}$	0.28
70:30 N_2:CO_2	$4.41^{A/A}$	0.21	$4.13^{A/AB}$	0.31	$4.26^{A/ABC}$	0.31	$4.06^{A/ABC}$	0.21	$4.12^{A/ABC}$	0.24
90:10 N_2:CO_2	$4.33^{A/A}$	0.26	$4.30^{A/A}$	0.33	$4.22^{A/AB}$	0.26	$4.05^{A/AB}$	0.26	$3.98^{A/AB}$	0.31
80:20 O_2:N_2	$4.29^{A/A}$	0.31	$4.02^{A/AB}$	0.29	$4.07^{A/AB}$	0.32	$3.90^{A/B}$	0.25	$3.85^{A/BC}$	0.28
40:30:30 CO_2:O_2:N_2	$4.22^{A/A}$	0.18	$4.14^{A/AB}$	0.19	$4.24^{A/A}$	0.31	$3.99^{A/AB}$	0.27	$4.02^{A/AB}$	0.24
Treatment/storage (days)	*Campylobacter* counts (log_{10} CFU/cm^2)									
	7	S.E	9	S.E	11	S.E	14	S.E	17	S.E
Control (air)	$3.72^{A/CD}$	0.32	$3.39^{AB/DE}$	0.39	$3.14^{A/E}$	0.53	$3.18^{A/E}$	0.37	$2.99^{A/E}$	0.55
10:90 N_2:CO_2	$4.01^{A/ABC}$	0.27	$4.06^{AB/AB}$	0.23	$3.91^{AB/BC}$	0.22	$3.90^{A/BC}$	0.25	$3.65^{AB/C}$	0.25
30:70 N_2:CO_2	$4.19^{A/AB}$	0.33	$4.02^{AB/BC}$	0.29	$3.68^{AB/CD}$	0.29	$3.61^{A/D}$	0.27	$3.59^{AB/D}$	0.21
50:50 N_2:CO_2	$4.17^{A/AB}$	0.24	$4.15^{B/AB}$	0.18	$4.15^{B/AB}$	0.29	$3.93^{A/B}$	0.34	$3.88^{AB/B}$	0.31
70:30 N_2:CO_2	$4.09^{A/ABC}$	0.27	$3.88^{AB/BC}$	0.25	$3.89^{AB/BC}$	0.30	$3.83^{A/C}$	0.37	$3.96^{B/BC}$	0.37
90:10 N_2:CO_2	$3.95^{A/AB}$	0.29	$4.01^{AB/AB}$	0.34	$3.87^{AB/B}$	0.31	$3.90^{A/B}$	0.28	$3.85^{AB/B}$	0.38
80:20 O_2:N_2	$3.47^{A/CD}$	0.33	$3.23^{A/DE}$	0.36	$3.17^{A/DE}$	0.34	$3.15^{A/DE}$	0.24	$3.09^{AB/E}$	0.33
40:30:30 CO_2:O_2:N_2	$3.77^{A/BC}$	0.32	$3.53^{AB/CD}$	0.22	$3.27^{AB/C}$	0.32	$3.18^{A/C}$	0.30	$3.19^{AB/C}$	0.37

[a] S.E. = standard error; X/Y, X = Comparisons were made between treatments for a sampling stage, Y = Comparisons made between days. The same letter indicates not statistically different at the 5% level ($P > 0.05$).

Table 2
Mean TVC mesophile counts (log_{10} CFU/cm^2) on chicken fillets packed in different MAP gaseous combinations over the course of 17 days storage at 2 °C.

Treatment/storage (days)	TVC mesophile counts (log_{10} CFU/cm^2)									
	1	S.E[a]	2	S.E	3	S.E	4	S.E	5	S.E
Control (air)	$2.82^{A/A}$	0.19	$3.23^{A/A}$	0.06	$4.07^{A/AB}$	0.34	$4.82^{B/B}$	0.14	$5.22^{CD/B}$	0.32
10:90 N_2:CO_2	$2.33^{A/A}$	0.13	$2.74^{A/A}$	0.22	$2.97^{A/A}$	0.18	$2.91^{A/A}$	0.23	$3.15^{AB/A}$	0.20
30:70 N_2:CO_2	$2.85^{A/A}$	0.46	$2.73^{A/A}$	0.19	$2.90^{A/A}$	0.13	$2.95^{A/A}$	0.12	$2.87^{A/A}$	0.35
50:50 N_2:CO_2	$2.54^{A/A}$	0.10	$2.63^{A/A}$	0.16	$2.91^{A/A}$	0.10	$3.21^{AB/AB}$	0.06	$3.13^{AB/AB}$	0.27
70:30 N_2:CO_2	$3.17^{A/AB}$	0.29	$2.92^{A/AB}$	0.32	$2.71^{A/A}$	0.17	$3.72^{AB/AB}$	0.21	$3.65^{ABC/AB}$	0.45
90:10 N_2:CO_2	$3.09^{A/A}$	0.15	$3.18^{A/A}$	0.28	$3.40^{A/AB}$	0.33	$4.50^{AB/BC}$	0.08	$5.32^{D/CD}$	0.42
80:20 O_2:N_2	$2.73^{A/A}$	0.15	$3.83^{A/AB}$	0.45	$4.00^{A/AB}$	0.03	$4.75^{B/BC}$	0.12	$5.78^{D/CD}$	0.75
40:30:30 CO_2:O_2:N_2	$2.64^{A/A}$	0.20	$2.71^{A/A}$	0.14	$2.99^{A/A}$	0.28	$3.25^{AB/A}$	0.20	$4.65B^{CD/B}$	0.42
Treatment/storage (days)	TVC mesophile counts (log_{10} CFU/cm^2)									
	7	S.E	9	S.E	11	S.E	14	S.E	17	S.E
Control (air)	$6.77^{C/C}$	0.07	$9.23^{C/D}$	0.07	$10.70^{C/E}$	0.66	$10.84^{C/E}$	0.38	$10.26^{E/DE}$	0.25
10:90 N_2:CO_2	$4.84^{AB/B}$	0.43	$6.23^{AB/C}$	0.30	$6.25^{AB/C}$	0.34	$6.01^{A/BC}$	0.29	$5.41^{AB/BC}$	0.12
30:70 N_2:CO_2	$3.61^{A/A}$	0.58	$5.65^{A/B}$	0.50	$5.36^{A/B}$	0.55	$5.88^{A/B}$	0.37	$5.15^{A/B}$	0.25
50:50 N_2:CO_2	$4.27^{A/BC}$	0.60	$5.40^{A/CD}$	0.55	$6.29^{AB/D}$	0.40	$6.20^{A/D}$	0.11	$5.76^{AB/D}$	0.69
70:30 N_2:CO_2	$4.17^{A/B}$	0.39	$5.48^{A/C}$	0.17	$6.98^{AB/D}$	0.42	$7.11^{AB/D}$	0.13	$6.95^{BC/D}$	0.37
90:10 N_2:CO_2	$5.99^{BC/D}$	0.52	$7.67^{BC/E}$	0.55	$7.85^{BC/E}$	0.37	$8.39^{B/E}$	0.44	$7.76^{CD/E}$	0.13
80:20 O_2:N_2	$6.64^{C/D}$	0.68	$8.04^{C/E}$	0.59	$9.41^{C/F}$	0.19	$10.79^{C/G}$	0.27	$9.20^{DE/EF}$	0.85
40:30:30 CO_2:O_2:N_2	$4.63^{AB/B}$	0.65	$5.45^{A/BC}$	0.49	$6.20^{A/C}$	0.17	$6.53^{A/C}$	0.21	$6.46^{ABC/C}$	0.41

[a] S.E. = standard error; X/Y, X = Comparisons were made between treatments for a sampling stage, Y = Comparisons made between days. The same letter indicates not statistically different at the 5% level ($P > 0.05$).

With TEC the effect of 40:30:30% CO_2:O_2:N_2 was observed more quickly as fillets packed in this MAP treatment showed significantly ($P < 0.05$) reduced growth after 5 days and 3 log_{10} cfu per cm^2 less of growth after 17 days (Table 4). As with TVC, the most effective treatments at inhibiting TEC were those that contained CO_2 with N_2 filler. Significant growth inhibition was obtained after 3 days with 70% CO_2 and 7 days with concentrations of 30% or higher.

Data for the spoilage organisms, lactic acid bacteria (LAB) and *Pseudomonas* are shown in Tables 5 and 6, respectively. Overall treatments with 50% or more CO_2 inhibited the growth of LAB but the effect was not as clear cut as was observed with TVC and TEC and after 17 days there was no significant difference between the control and 50:50% N_2:CO_2 counts. Furthermore, most samples from 70:30% N_2:CO_2, 90:10% N_2:CO_2 and 80:20% O_2:N_2 packs showed LAB counts that were similar or higher than the control counts. After 5 days, all treatments, with the exception of 90:10% N_2:CO_2 and 80:20% O_2:N_2 showed reduced *Pseudomonas* growth (Table 6). After 9 days only the latter was statistically similar to the control counts. By the end of the storage period, MAP treatments with 30% CO_2 or higher with N_2 making up the remaining gas were approximately 4.5–6.0 log_{10} CFU/cm^2 lower than the control counts. Overall these treatments extended the shelf-life of fresh chicken fillets from approximately 7 days to in excess of 17 days.

While the presence of CO_2 inhibited TVC, TEC, LAB and Pseudomonas growth, for the majority of samples, this effect was not statistically significant ($P > 0.05$) until after 5 days of storage after which time the concentration of CO_2 (ppm) in the chicken fillets was less than 250 ppm in control, 80:20% O_2:N_2 and 90:10% N_2:CO_2 packs, 500–1000 ppm in 70:30% N_2:CO_2, 40:30:30% CO_2:O_2:N_2 and 50:50% N_2:CO_2 packs, 1000–1500 ppm for 30:70% N_2:CO_2 and up to 2000 ppm in 10:90% N_2:CO_2 packs (Fig. 1). In contrast the concentration of O_2 dissolved in the fillets decreased from 34 to 0 ppm over the 17 day duration of the experiment in 80:20% O_2:N_2 packs; from 9 to 0 ppm in the control samples over 8 days and from

Table 3
Mean TVC psychrophilic counts (log_{10} CFU/cm^2) on chicken fillets packed in different MAP gaseous combinations over the course of 17 days storage at 2 °C.

Treatment/storage (days)	TVC psychrophile counts (log_{10} CFU/cm^2)									
	1	S.E[a]	2	S.E	3	S.E	4	S.E	5	S.E
Control (air)	$2.99^{A/A}$	0.18	$3.31^{AB/A}$	0.14	$3.89^{A/AB}$	0.47	$4.72^{C/B}$	0.13	$4.75^{BC/B}$	0.62
10:90 N_2:CO_2	$2.45^{A/A}$	0.26	$2.83^{AB/A}$	0.20	$2.49^{A/A}$	0.47	$2.96^{A/A}$	0.24	$2.89^{A/A}$	0.39
30:70 N_2:CO_2	$3.11^{A/AB}$	0.37	$2.37^{A/A}$	0.33	$2.72^{A/AB}$	0.21	$3.00^{A/AB}$	0.10	$3.02^{A/AB}$	0.30
50:50 N_2:CO_2	$2.66^{A/A}$	0.18	$2.64^{AB/A}$	0.14	$2.73^{A/AB}$	0.14	$3.03^{AB/AB}$	0.08	$3.11^{A/AB}$	0.31
70:30 N_2:CO_2	$3.12^{A/AB}$	0.37	$2.92^{AB/A}$	0.31	$2.80^{A/A}$	0.14	$3.66^{ABC/AB}$	0.24	$3.63^{AB/AB}$	0.42
90:10 N_2:CO_2	$3.04^{A/A}$	0.14	$3.05^{AB/A}$	0.35	$3.50^{A/AB}$	0.31	$4.50^{BC/BC}$	0.05	$5.08^{BC/CD}$	0.25
80:20 O_2:N_2	$2.85^{A/A}$	0.06	$3.90^{B/AB}$	0.43	$3.95^{A/AB}$	0.03	$4.59^{C/ABC}$	0.08	$5.51^{C/BC}$	0.62
40:30:30 CO_2:O_2:N_2	$2.56^{A/AB}$	0.14	$2.27^{A/A}$	0.35	$2.81^{A/AB}$	0.30	$3.25^{ABC/AB}$	0.15	$3.69^{AB/B}$	0.61
Treatment/storage (days)	TVC psychrophile counts (log_{10} CFU/cm^2)									
	7	S.E	9	S.E	11	S.E	14	S.E	17	S.E
Control (air)	$6.83^{D/C}$	0.15	$8.69^{C/D}$	0.27	$8.76^{C/D}$	0.93	$11.15^{C/E}$	0.44	$11.04^{C/E}$	0.07
10:90 N_2:CO_2	$5.15^{ABC/B}$	0.31	$6.30^{AB/B}$	0.34	$5.25^{A/B}$	0.29	$6.12^{A/B}$	0.49	$6.14^{A/B}$	0.30
30:70 N_2:CO_2	$3.75^{A/B}$	0.65	$5.57^{A/C}$	0.49	$5.12^{A/C}$	0.52	$5.61^{A/C}$	0.08	$5.83^{A/C}$	0.25
50:50 N_2:CO_2	$3.92^{A/BC}$	0.40	$5.09^{A/CD}$	0.40	$6.28^{AB/DE}$	0.55	$6.33^{AB/E}$	0.34	$6.73^{A/E}$	0.12
70:30 N_2:CO_2	$4.24^{AB/B}$	0.34	$5.56^{A/C}$	0.15	$7.13^{B/D}$	0.30	$6.85^{AB/D}$	0.14	$7.22^{AB/D}$	0.17
90:10 N_2:CO_2	$5.71^{BCD/D}$	0.17	$7.74^{BC/E}$	0.57	$7.56^{BC/E}$	0.25	$7.64^{B/E}$	0.16	$8.49^{B/E}$	0.36
80:20 O_2:N_2	$6.16^{CD/C}$	0.35	$8.01^{C/D}$	0.36	$8.72^{C/D}$	0.25	$10.17^{C/E}$	0.32	$10.79^{C/E}$	0.33
40:30:30 CO_2:O_2:N_2	$5.04^{ABC/C}$	0.95	$5.57^{A/CD}$	0.52	$6.24^{AB/CDE}$	0.30	$6.47^{AB/DE}$	0.18	$7.16^{AB/E}$	0.12

[a] S.E. = standard error; X/Y, X = Comparisons were made between treatments for a sampling stage, Y = Comparisons made between days. The same letter indicates not statistically different at the 5% level ($P > 0.05$).

Table 4
Mean total *Enterobacteriaceae* counts (TEC, log_{10} CFU/cm^2) on chicken fillets packed in different MAP gaseous combinations over the course of 17 days storage at 2 °C.

Treatment/storage (days)	Total *Enterobacteriaceae* counts (log_{10} CFU/cm^2)									
	1	S.E[a]	2	S.E	3	S.E	4	S.E	5	S.E
Control (air)	$1.83^{A/A}$	0.29	$2.21^{A/A}$	0.12	$3.58^{B/B}$	0.37	$2.67^{BC/AB}$	0.41	$3.88^{CD/BC}$	0.14
10:90 N_2:CO_2	$1.69^{A/A}$	0.17	$2.20^{A/AB}$	0.12	$2.26^{AB/AB}$	0.48	$1.25^{A/A}$	0.54	$1.26^{A/A}$	0.24
30:70 N_2:CO_2	$2.08^{A/ABC}$	0.43	$1.53^{A/A}$	0.06	$2.43^{AB/ABC}$	0.23	$1.71^{ABC/AB}$	0.26	$2.10^{AB/ABC}$	0.32
50:50 N_2:CO_2	$1.50^{A/A}$	0.19	$1.89^{A/AB}$	0.19	$2.45^{AB/ABC}$	0.17	$1.62^{ABC/A}$	0.59	$2.24^{AB/AB}$	0.12
70:30 N_2:CO_2	$2.05^{A/A}$	0.37	$2.21^{A/A}$	0.07	$2.19^{A/A}$	0.16	$2.02^{ABC/A}$	0.39	$2.78^{BC/AB}$	0.22
90:10 N_2:CO_2	$1.67^{A/A}$	0.23	$2.06^{A/AB}$	0.13	$3.16^{AB/BCD}$	0.20	$2.96^{C/BC}$	0.68	$4.15^{D/CD}$	0.23
80:20 O_2:N_2	$1.47^{A/A}$	0.51	$2.27^{A/AB}$	0.19	$3.11^{AB/BC}$	0.16	$2.01^{ABC/AB}$	0.58	$3.85^{CD/CD}$	0.18
40:30:30 CO_2:O_2:N_2	$1.53^{A/AB}$	0.15	$1.95^{A/AB}$	0.05	$2.73^{AB/BC}$	0.17	$1.39^{AB/A}$	0.48	$2.22^{AB/ABC}$	0.06
Treatment/storage (days)	Total *Enterobacteriaceae* counts (log_{10} CFU/cm^2)									
	7	S.E	9	S.E	11	S.E	14	S.E	17	S.E
Control (air)	$5.07^{C/CD}$	0.16	$5.92^{C/DE}$	0.54	$6.52^{D/E}$	0.74	$7.82^{D/F}$	0.60	$8.45^{D/F}$	0.45
10:90 N_2:CO_2	$3.26^{AB/B}$	0.46	$3.10^{AB/B}$	0.40	$3.42^{AB/B}$	0.30	$3.47^{AB/B}$	0.59	$5.17^{AB/C}$	0.32
30:70 N_2:CO_2	$3.00^{AB/CD}$	0.29	$4.04^{B/DE}$	0.28	$2.87^{A/BCD}$	0.28	$2.36^{A/ABC}$	0.40	$4.31^{A/E}$	0.36
50:50 N_2:CO_2	$3.05^{AB/BCD}$	0.45	$3.08^{AB/BCD}$	0.27	$3.67^{AB/CDE}$	0.28	$4.08^{B/DE}$	0.16	$4.81^{A/E}$	0.19
70:30 N_2:CO_2	$3.10^{AB/ABC}$	0.32	$3.53^{AB/BC}$	0.33	$4.09^{B/C}$	0.65	$5.55^{CD/D}$	0.11	$6.21^{BC/D}$	0.21
90:10 N_2:CO_2	$4.27^{BC/D}$	0.13	$6.14^{C/E}$	0.14	$6.61^{D/EF}$	0.24	$7.31^{D/EF}$	0.20	$7.85^{D/F}$	0.38
80:20 O_2:N_2	$4.37^{BC/CD}$	0.16	$5.71^{C/E}$	0.30	$5.09^{C/DE}$	0.34	$5.67^{CD/E}$	0.62	$7.43^{CD/F}$	0.22
40:30:30 CO_2:O_2:N_2	$2.70^{A/BC}$	0.32	$2.54^{A/ABC}$	0.18	$3.32^{AB/C}$	0.24	$3.23^{AB/C}$	0.22	$5.36^{AB/D}$	0.09

[a] S.E. = standard error; X/Y, X = Comparisons were made between treatments for a sampling stage, Y = Comparisons made between days. The same letter indicates not statistically different at the 5% level ($P > 0.05$).

0.4–0.5 ppm to 0 ppm over 1.7–2.8 days in 10:90% 30:70% 50:50% 70:30% and 90:10% N_2:CO_2 packs (Fig. 2). Interestingly the dissolved O_2 concentration in 40:30:30% CO_2:O_2:N_2 packed chicken fillets remained at 11 ppm throughout the experiment.

4. Discussion

Campylobacter declined more quickly in the presence of O_2. Although *Campylobacter* respond to atmospheric oxygen exposure using an antioxidant defence system composed of superoxide dismutase, catalase or glutathione peroxidise activity (Storz and Imlay, 1999), which may result in long term aerobic adaptation (Jones et al., 1993; Harvey and Leach, 1998; Klancnik et al., 2009), our results suggest oxidative stress is still detrimental for the survival of at least a sub-population of cells. The decrease in *Campylobacter* levels observed in the 80:20% O_2:N_2 samples after 17 days of 1.26 log_{10} was similar to the 1.2 log_{10} reduction reported by Rajkovic et al. (2010) but less than the 2.2 to 3.1 log_{10} achieved by Boysen et al. (2007) using 70:30% O_2:CO_2. In direct contrast to the effect of oxygen, CO_2 in the MAP mixture apparently assisted the survival of *Campylobacter*, an effect that has also been previously reported (Boysen et al., 2007). This may be attributed to the inhibition of other organisms in gaseous mixtures containing CO_2 as under normal atmospheric conditions *Campylobacter* are poor competitors (Huat et al., 2010).

CO_2 inhibited the growth of TVC, TEC, LAB and *Pseudomonas* but only at concentrations of 50–90% where a shelf-life in excess of 17 days at 2 °C was obtained. Reduced bacterial growth in CO_2 packs is well documented (Gill et al., 1990) as is the concentration dependence of this effect (Patsias et al., 2006b). Indeed, Stiles (1991a,b), previously reported that a minimum CO_2 concentration of 20–30% is required before an inhibitory effect is observed which is reasonably consistent with our observations. Based on a TVC mesophile count of 7 log_{10} CFU cm^{-2} as an indication of the end of shelf-life (ICMSF, 1986), the shelf-life of our fillets packed in air was 7 days which was extended to 11 days with 70:30% N_2:CO_2 and in

Table 5
Mean lactic acid bacteria (LAB) counts (log_{10} CFU/cm^2) on chicken fillets packed in different MAP gaseous combinations over the course of 17 days storage at 2 °C.

Treatment/storage (days)	Lactic acid bacteria (LAB) counts (log_{10} CFU/cm^2)									
	1	S.E[a]	2	S.E	3	S.E	4	S.E	5	S.E
Control	$1.68^{AB/A}$	0.56	$1.94^{AB/AB}$	0.10	$2.23^{BC/BC}$	0.26	$2.65^{CD/CD}$	0.13	$2.85^{BC/D}$	0.18
10:90 N_2:CO_2	$1.69^{AB/A}$	0.16	$2.00^{AB/AB}$	0.23	$2.07^{ABC/AB}$	0.26	$1.79^{A/AB}$	0.14	$2.17^{A/B}$	0.26
30:70 N_2:CO_2	$1.88^{ABCD/ABC}$	0.31	$1.60^{A/A}$	0.37	$1.85^{AB/AB}$	0.17	$2.09^{AB/BC}$	0.08	$2.29^{A/CD}$	0.19
50:50 N_2:CO_2	$1.53^{A/A}$	0.27	$1.99^{AB/BC}$	0.12	$1.76^{A/AB}$	0.13	$2.24^{BC/C}$	0.12	$2.21^{A/C}$	0.24
70:30 N_2:CO_2	$2.05^{BCD/A}$	0.24	$1.86^{AB/A}$	0.17	$1.93^{AB/A}$	0.08	$2.61^{CD/B}$	0.02	$2.91^{CD/B}$	0.26
90:10 N_2:CO_2	$2.26^{D/B}$	0.17	$1.58^{A/A}$	0.43	$2.20^{BC/B}$	0.25	$2.81^{D/C}$	0.19	$3.61^{E/D}$	0.34
80:20 O_2:N_2	$2.16^{CD/A}$	0.14	$2.51^{C/A}$	0.22	$2.49^{C/A}$	0.09	$2.55^{CD/A}$	0.12	$3.35^{DE/B}$	0.27
40:30:30 CO_2:O_2:N_2	$1.81^{ABC/A}$	0.26	$2.10^{BC/AB}$	0.04	$2.16^{ABC/AB}$	0.20	$2.05^{AB/AB}$	0.26	$2.48^{AB/B}$	0.12
Treatment/storage (days)	Lactic acid bacteria (LAB) counts (log_{10} CFU/cm^2)									
	7	S.E	9	S.E	11	S.E	14	S.E	17	S.E
Control	$4.18^{D/E}$	0.21	$5.43^{E/F}$	0.10	$6.15^{D/G}$	0.22	$6.51^{E/G}$	0.29	$6.49^{BC/G}$	0.13
10:90 N_2:CO_2	$2.91^{AB/C}$	0.08	$3.45^{A/D}$	0.05	$3.68^{A/D}$	0.16	$4.14^{A/E}$	0.43	$5.08^{A/F}$	0.37
30:70 N_2:CO_2	$2.56^{A/D}$	0.17	$3.78^{AB/E}$	0.12	$4.29^{AB/F}$	0.14	$4.38^{A/F}$	0.24	$5.38^{A/G}$	0.20
50:50 N_2:CO_2	$2.84^{AB/D}$	0.16	$4.00^{AB/E}$	0.04	$4.85^{BC/F}$	0.42	$5.57^{BC/G}$	0.13	$5.93^{B/G}$	0.08
70:30 N_2:CO_2	$3.40^{C/C}$	0.21	$4.60^{BC/D}$	0.12	$5.45^{CD/E}$	0.10	$6.06^{D/F}$	0.11	$6.42^{CD/F}$	0.08
90:10 N_2:CO_2	$4.25^{D/E}$	0.16	$4.94^{CD/F}$	0.29	$5.83^{D/G}$	0.12	$5.96^{CD/G}$	0.05	$6.94^{D/H}$	0.53
80:20 O_2:N_2	$4.08^{D/C}$	0.10	$5.37^{DE/D}$	0.05	$5.43^{CD/D}$	0.15	$6.29^{DE/E}$	0.29	$6.78^{CD/F}$	0.18
40:30:30 CO_2:O_2:N_2	$3.21^{BC/C}$	0.24	$3.95^{AB/D}$	0.16	$4.74^{BC/E}$	0.44	$5.33^{B/F}$	0.15	$6.25^{BC/G}$	0.21

[a] S.E. = standard error; X/Y, X = Comparisons were made between treatments for a sampling stage, Y = Comparisons made between days. The same letter indicates not statistically different at the 5% level ($P > 0.05$).

excess of 17 days with CO_2 concentrations higher than 50%. Chouliara et al. (2007) observed a 6 day shelf-life in air packs which was extended by 3 and 7 days using 30:70% and 70:30% CO_2:N_2, respectively. Boysen et al. (2007) also found higher CO_2 concentrations were more effective at inhibiting bacterial growth on raw poultry and reported a shelf-life in excess of 20 days at 4 °C with 60:40% and 90:10% CO_2:N_2.

After 9 days at 2 °C the *Pseudomonas* counts were 3.7–4.5 log_{10} CFU/cm^2 lower in packs containing 30–50% CO_2, considerably higher than the 1.1–2.1 log_{10} CFU/cm^2 reduction previously reported by Chouliara et al. (2007) for raw poultry stored at 4 °C under the same atmospheric conditions. Regardless, these studies show that gaseous combinations of CO_2 and N_2 without O_2 significantly inhibit the growth of *Pseudomonas*, the main spoilage organisms in meat (Jay, 1986). This study therefore provides more evidence that CO_2 retards the growth of aerobic spoilage bacteria as a result of an extension of the lag phase and a decreased growth rate during the logarithmic phase of growth as has been previously suggested (Farber, 1991). Indeed if 10^{7-8} CFU/cm^{-2} *Pseudomonas* was used as the indicator of the end of shelf-life as suggested by Nychas et al. (2008) and Charles et al. (2006), all of the packs with 30% or higher CO_2 concentrations showed a shelf-life extension in excess of 10 days. This effect has been attributed to *Pseudomonas* being strict aerobes, thus requiring O_2 for metabolism and growth. However, significant growth was still observed in this and other studies (Patsias et al., 2006a,b) in packs without added O_2, suggesting the transmission of O_2, all-be-it at low levels, across the packaging film. It is also worth noting that low O_2 concentrations were not the only factor inhibiting *Pseudomonas* growth in our study as fillets packed in 40:30:30% CO_2:O_2:N_2 had significantly lower counts from 4 days onwards as compared to the control packs.

To the best of our knowledge this is the first study to measure the concentration of dissolved CO_2 in chicken fillets using a range of different gaseous combinations and over time during chilled storage. In packs with a CO_2 concentration of 30% or higher, most of the dissolution into the chicken fillet occurred in the first 24 h although

Table 6
Mean *Pseudomonas* counts (log_{10} CFU/cm^2) on chicken fillets packed in different MAP gaseous combinations over the course of 17 days storage at 2 °C.

Treatment/storage (days)	*Pseudomonas* counts (log_{10} CFU/cm^2)									
	1	S.E[a]	2	S.E	3	S.E	4	S.E	5	S.E
Control	$2.71^{A/A}$	0.15	$3.53^{A/AB}$	0.13	$4.07^{A/ABC}$	0.47	$4.87^{C/BC}$	0.12	$5.37^{C/CD}$	0.32
10:90 N_2:CO_2	$2.19^{A/A}$	0.14	$2.85^{A/A}$	0.12	$2.38^{A/A}$	0.34	$2.77^{A/A}$	0.27	$2.16^{A/A}$	0.84
30:70 N_2:CO_2	$2.57^{A/A}$	0.49	$2.11^{A/A}$	0.30	$2.42^{A/A}$	0.25	$2.82^{A/A}$	0.18	$2.67^{A/A}$	0.42
50:50 N_2:CO_2	$2.07^{A/A}$	0.10	$2.39^{A/A}$	0.04	$2.53^{A/A}$	0.18	$2.89^{AB/A}$	0.11	$2.70^{A/A}$	0.27
70:30 N_2:CO_2	$2.90^{A/A}$	0.40	$3.00^{A/A}$	0.21	$2.32^{A/A}$	0.21	$3.38^{ABC/AB}$	0.23	$3.22^{AB/A}$	0.34
90:10 N_2:CO_2	$2.70^{A/A}$	0.18	$3.08^{A/AB}$	0.16	$3.34^{A/ABC}$	0.36	$4.22^{ABC/BCD}$	0.16	$4.87^{BC/CD}$	0.23
80:20 O_2:N_2	$2.50^{A/A}$	0.09	$3.35^{A/AB}$	0.06	$4.11^{A/BC}$	0.27	$4.47^{BC/BC}$	0.15	$5.47^{C/DE}$	0.62
40:30:30 CO_2:O_2:N_2	$2.26^{A/A}$	0.22	$2.83^{A/A}$	0.07	$2.62^{A/A}$	0.26	$3.13^{AB/A}$	0.17	$3.38^{AB/AB}$	0.70
Treatment/storage (days)	*Pseudomonas* counts (log_{10} CFU/cm^2)									
	7	S.E	9	S.E	11	S.E	14	S.E	17	S.E
Control	$6.83^{C/D}$	0.12	$9.25^{D/E}$	0.07	$9.83^{D/EF}$	0.18	$11.15^{C/F}$	0.50	$10.49^{D/EF}$	0.27
10:90 N_2:CO_2	$4.74^{AB/B}$	0.41	$6.27^{AB/C}$	0.35	$5.47^{AB/BC}$	0.15	$6.11^{AB/BC}$	0.43	$5.95^{AB/BC}$	0.34
30:70 N_2:CO_2	$3.57^{A/AB}$	0.72	$5.59^{A/C}$	0.49	$5.28^{A/C}$	0.57	$4.54^{A/BC}$	0.67	$4.80^{A/BC}$	0.51
50:50 N_2:CO_2	$3.55^{A/AB}$	0.58	$4.65^{A/BC}$	0.42	$4.98^{A/BC}$	0.40	$5.11^{A/C}$	0.31	$4.53^{A/BC}$	0.28
70:30 N_2:CO_2	$3.40^{A/AB}$	0.33	$4.78^{A/BC}$	0.24	$5.13^{A/C}$	0.24	$5.39^{A/C}$	0.13	$5.77^{AB/C}$	0.07
90:10 N_2:CO_2	$5.25^{BC/D}$	0.22	$7.54^{BC/E}$	0.63	$6.98^{BC/E}$	0.26	$7.16^{B/E}$	0.29	$7.77^{C/E}$	0.54
80:20 O_2:N_2	$6.31^{BC/E}$	0.47	$8.04^{CD/F}$	0.58	$8.47^{CD/FG}$	0.30	$9.98^{C/GH}$	0.32	$10.54^{D/H}$	0.50
40:30:30 CO_2:O_2:N_2	$4.90^{AB/BC}$	0.97	$4.99^{A/C}$	0.61	$5.17^{A/CD}$	0.15	$5.31^{A/CD}$	0.51	$6.56^{BC/D}$	0.31

[a] S.E. = standard error; X/Y, X = Comparisons were made between treatments for a sampling stage, Y = Comparisons made between days. The same letter indicates not statistically different at the 5% level ($P > 0.05$).

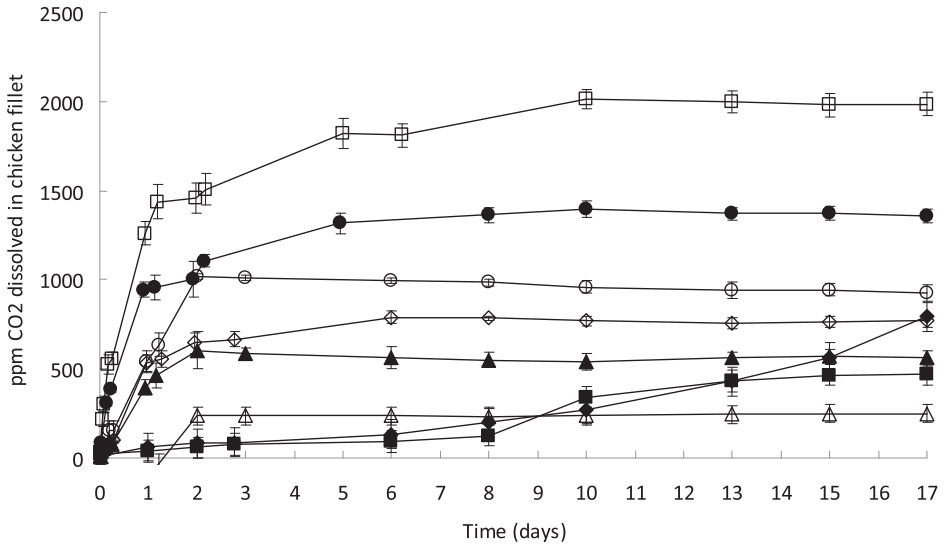

Fig. 1. Dissolved CO_2 (ppm) in poultry fillets stored at 2 °C over 17 days in modified atmospheres; with (■ control, ▫10:90% N_2:CO_2; ●30:70% N_2:CO_2; ⁘50:50% N_2:CO_2; ▲70:30% N_2:CO_2; △ 90:10% N_2:CO_2; ◆80:20% O_2:N_2; ◇ 40:30:30% CO_2:O_2:N_2).

it required 2–7 days before an equilibrium was achieved, at levels directly related to the original concentration of CO_2 in the pack. It has been previously established that CO_2 in MAP is absorbed by water and lipids in the food until an equilibrium or saturation is achieved (Jakobsen and Bertelsen, 2006). The high rate of CO_2 absorption into poultry during the first day's storage has been previously reported (Rotabakk et al., 2010; Al-Nehlawi et al., 2013). Al-Nehlawi et al. (2013) observed an average of 567 ppm CO_2 dissolved into a chicken drumstick after 24 h in packs containing 70:15:15% CO_2:O_2:N_2 and stored at 3 °C, which is similar to our observations with 40:30:30% CO_2:O_2:N_2. Rotabakk et al. (2010) obtained 450 ppm CO_2 in chicken fillets after 120 min in 100% CO_2, well within the range of 303 ppm (102 min) and 525 (257 min) observed in this study with 10:90%N_2:CO_2.

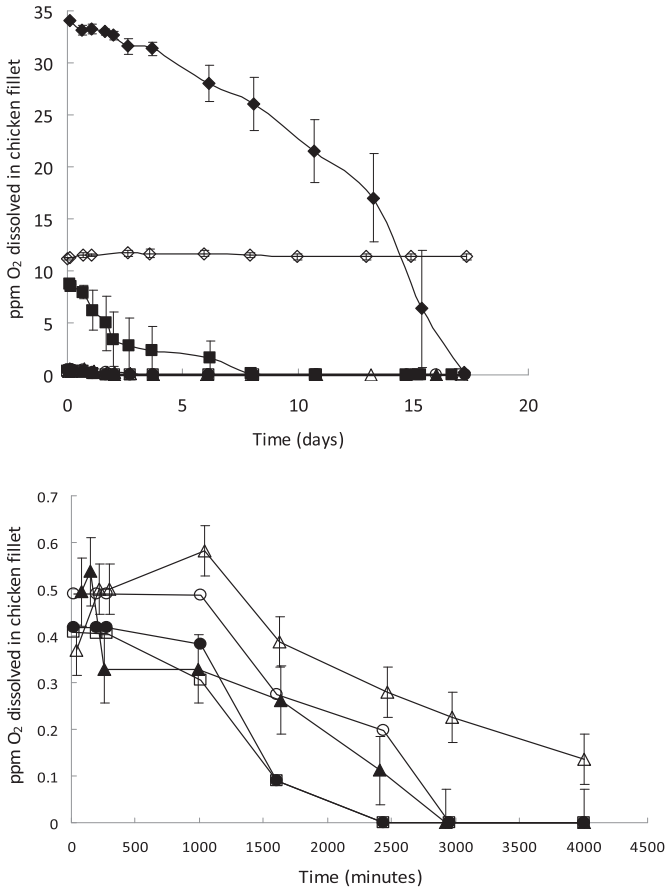

Fig. 2. Dissolved O_2 (ppm) in poultry fillets stored at 2 °C over 17 days in modified atmospheres; with (■ control, ▫10:90% N_2:CO_2; ●30:70% N_2:CO_2; ⁘50:50% N_2:CO_2; ▲70:30% N_2:CO_2; △ 90:10% N_2:CO_2; ◆ 80:20% O_2:N_2; ◇ 40:30:30% CO_2:O_2:N_2).

Using the volumetric method to estimate the amount of dissolved CO_2 in semi rigid trays has some limitations (Rotabakk et al., 2007) as the semi-rigid trays do not allow for an exact measurement of the amount of gases in the atmosphere because part of the decrease on partial pressure is absorbed by the rigid behaviour of the package. Thus changes in the volume are affected, leading to an underestimation of dissolved gas in the food product. To protect against this a g/p-ratio was 5:1 was used in the present studies and a second method (Henrys constant method) was also used to estimate the concentration of dissolved CO_2. The similarity of results (day 3 to day 5–6) obtained using the two methods suggested that using the higher g/p ratio was effective in overcoming any issues with tray rigidity.

There is a clear connection between the observed decrease in O_2 and corresponding increase in CO_2 in the packs with 80:20 O_2:N_2 (Figs. 1 and 2). The same phenomenon was also observed in the control packs. This is most likely caused by the growth of aerobic bacteria, resulting in the consumption of O_2 and the production of CO_2 (Boskou and Debevere, 1997; Fletcher et al., 2002). Although the changes in head space gas composition changed due to microbial metabolic activity, the poultry samples were still in equilibrium with the surrounding gas as described by Henry's law.

Enterobacteriaceae also grew on poultry fillets packed in the different atmospheric gaseous combinations but significantly reduced growth was observed in packs containing 30% or more CO_2. This slower rate of growth in the absence of O_2 has been previously reported on poultry (Chouliara et al., 2007) and is in agreement with the results of Rajkovic et al. (2010), Boysen et al. (2007) and Gill et al. (1990). This and the other data presented in this study support the application of gaseous mixtures containing 30–90% CO_2 with the balance as N_2 to inhibit bacteria growth and extend

the shelf-life of fresh chicken fillets. It also shows that these gaseous combinations support the survival of *Campylobacter* which is optimally reduced in packs containing air or high levels of O_2. However, a compromise mixture containing 40:30:30% CO_2:O_2:N_2 will achieve the extended shelf-life without promoting the maintenance of *Campylobacter* and this mixture should be used in the poultry industry.

5. Conclusions

Although the application of higher CO_2 concentrations in MAP increases shelf-life, 50% is as effective as 90% at controlling TVC, TEC and *Pseudomonas* on chicken fillets stored at 2 °C, while 70% was as effective as the higher concentration for LAB. 40:30:30 CO_2:O_2:N_2, was the most appropriate gaseous mixture for achieving the dual objectives of extending shelf-life while inhibiting *Campylobacter* survival. The data presented in this and related studies should provide the basis for new microbial models that will predict microbial growth and shelf-life of poultry fillets stored under different MAP conditions thus facilitating a more scientific approach to pathogen control and reduced waste in the poultry industry.

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